Pestic. Sci. 1997, **49**, 178–184

Accumulation and Elimination Kinetics of Herbicides Butachlor, Thiobencarb and Chlomethoxyfen by *Aristichthys nobilis*

Kuo-Hsiung Lin, Jui-Hung Yen & Yei-Shung Wang*

Laboratory of Pesticide Chemistry, Department of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan

(Received 19 January 1996; accepted 20 September 1996)

Abstract: Bioconcentration kinetics of three major paddy-field herbicides, butachlor, thiobencarb and chlomethoxyfen were investigated by accumulation and elimination measurements in laboratory experiments with black silver carp (*Aristichthys nobilis*). The biological half-lives of the three herbicides on exposure at high and low concentrations were 11·6 and 23·1 days for butachlor, 13·9 and 17·3 days for thiobencarb and 5·6 and 4·5 h for chlomethoxyfen, respectively. One- and two-compartment models were used to elucidate the bioconcentration kinetics. Data from a short-term, 14-day expiry experiment were used to estimate the parameters in the models by non-linear regression analysis, and the bioconcentration factors (BCF) in the steady state were calculated from those parameters. The BCF in the steady state have the descending order thiobencarb > chlomethoxyfen > butachlor at high concentration, and butachlor > thiobencarb > chlomethoxyfen at low concentration. One-compartment model for butachlor and thiobencarb and a two-compartment model for chlomethoxyfen are useful to predict the BCF on prolonged exposure and, further, to assess the influence of these pollutants.

Key words: accumulation, elimination, butachlor, thiobencarb, chlomethoxyfen, *Aristichthys nobilis*

1 INTRODUCTION

Butachlor, thiobencarb and chlomethoxyfen are the most popular herbicides used to control weeds in paddy fields in Taiwan. After application to the paddy, the herbicides dissipate in paddy water and are adsorbed on the soil.[1–3] They disappear in irrigation water,[4] but minor amounts of the herbicides can still be detected in paddy drainage water several weeks after application.[5] Herbicides applied to paddy fields may also flow out with irrigated water, causing contamination of river water.[6–8]

Estimation of the bioaccumulation potential in fish is important to evaluate possible environmental hazards from chemicals. Fish take up lipophilic compounds from water via their gills with oxygen,[9,10] and high concentrations in body fat can eventually be attained by partitioning.[11]

An accelerated bioconcentration test procedure, based on the kinetics of a one-compartment system has been developed by Branson *et al.*[12] using 2,2′,4,4′-tetrachlorobiphenyl as a model compound. They used non-linear regression analysis to estimate the rate coefficients for uptake and clearance, and the bioconcentration factor in the steady state was calculated from the rate coefficients. A two-compartment model was used to describe the kinetics of chemicals in guppies by Könemann and Leeuwen.[13] They determined the rate coefficients for uptake and elimination of six chlorobenzene compounds and their bioaccumulation.

In this work we used a two-compartment model to describe the kinetics of three herbicides—butachlor,

* To whom correspondence should be addressed.

Pestic. Sci. 0031-613X/97/$09.00

thiobencarb and chlomethoxyfen—in black silver carp (*Aristichthys nobilis*) and compared the results to those of a one-compartment model. The kinetics of uptake and elimination in fish, the kinetic parameters of the three herbicides in an individual model and the prediction of bioconcentration in the steady state with an accelerated procedure were also examined.

2 MATERIALS AND METHODS

2.1 Chemicals

Analytical grades of butachlor (purity 99·2%, Monsanto Co., USA), thiobencarb (100%, Kumiai Chemical Industry Co., Ltd, Japan) and chlomethoxyfen (99·9%, Ishihara Sangyo Co., Ltd, Japan) were obtained as reference standards for analysis. Technical grade herbicides, (butachlor 90%, thiobencarb 93% and chlomethoxyfen 85%) were also provided by these companies.

2.2 Maintenance and testing of fish

Black silver carp (*A. nobilis*), size 4–6 cm and mass 3–5 g, cultivated at the Chu-Pei Fish Culture Station, Hsinchu, Taiwan, were used as test fish. The test fish were acclimatized for one week in conditions similar to those under which the tests were performed. They were fed once a day during the acclimation period and were not fed for a period two days prior to the exposure to herbicides.

Prolonged tests of bioconcentration were carried out in a constant-flow system. A constant-dosing apparatus consisted of an aquarium (50 litre), a chemical stock bottle and a micro-tube pump. Taipei tap water (pH: 6·6; DO: 4·9 mg litre^{-1}; hardness: 215 mg litre^{-1} as calcium carbonate was air-pumped for more than 2 h at a flow-rate of 20 litre min^{-1} before it was used as dilution water. The water temperature was kept constant at room temperature (26–28°C) and the oxygen concentration always exceeded 7 μl litre^{-1} during the experiment. A mixture of three herbicides, butachlor, thiobencarb and chlomethoxyfen, at 12·5, 25·0 and 87·5 μM (3·9, 6·5 and 26·6 mg), respectively, in acetone was used as stock solution for tests at a high concentration and at 2·5, 5·0 and 8·75 μM (0·78, 1·29 and 2·66 mg), respectively, for a low concentration. This stock solution of herbicides in acetone was introduced into and mixed with tap water; the herbicide concentrations were controlled by the flow-rate of the stock solution with a micro-tube pump. Each cycle (4 min) delivered water (1 litre) to an aquarium with flow of effluent (1 litre) from an upper hole of the aquarium. The relation between flow volume and fish mass was 1·0 litre g^{-1} fish day^{-1}. Influent herbicide solution was introduced to the aquarium one hour before the experiment started. Two nominal exposure concentrations of the three herbicides depended on sublethal dose, water solubility, and the analytical detection limits. The actual concentrations of the three herbicides in water at high and low concentration exposure were measured every other day during the exposure period (Table 1).

Into two aquaria containing mixed chlomethoxyfen, butachlor and thiobencarb at high and low concentrations were introduced 85 fish each for a bioconcentration experiment. The fish were fed during the experiment. Two fish were removed from each aquarium for analysis of accumulated herbicide at zero time exposure and at 1, 2, 4 and 5 days after the experiment started, and then at two or three-day intervals throughout the experiment. After exposure to chemicals for 14

TABLE 1
Sublethal Concentration and Water Solubility of Herbicides Butachlor, Thiobencarb and Chlomethoxyfen, and the Nominal and Measured Concentrations in Water during 34-day Exposure Experiments

			Concentration, C_1			
			High level		*Low level*	
Herbicide	LC_{50}[a]	*Water solubility (20°C)*	*Nominal*	*Measured (Average)*	*Nominal*	*Measured (Average)*
	mg litre^{-1}		μg litre^{-1}			
Butachlor	0·58	20[b]	3·12	2·81 (±0·2)	0·62	0·62 (±0·03)
Thiobencarb	2·45	30[c]	5·16	4·12 (±0·7)	1·03	1·03 (±0·05)
Chlomethoxyfen	>1·78	0·39[d]	21·28	17·9 (±2·6)	2·13	2·13 (±0·02)

[a] Data from Ref. 15.
[b] Data from Ref. 17.
[c] Data from Ref. 18.
[d] Data from Ref. 19.

days, 35 were transferred separately from each aquarium to another aquarium (same size) containing clean water for the depuration experiment. The remaining fish continued in the bioconcentration experiment through 34 days. The aquaria for the clearance experiment were equipped with a filter containing excess active carbon to adsorb herbicides or other organic molecules to keep the water clean. Two fish were removed from each treatment for chemical analysis at 1, 3, 8 and 19 hours and then at intervals of two or three days until 25 days after the depuration experiment started.

2.3 Chemical analysis

Fish samples were weighed, homogenized in hexane + acetonitrile (1 + 1 by volume; 50 ml) and filtered. The residue was washed twice with the same solvent and the mixed solvent was combined. The hexane layer of the mixed solvent was separated and extracted with acetonitrile (3 × 150 ml). The acetonitrile portion was collected and evaporated with a rotary evaporator to a small volume and passed through a column (6 cmx0·8 cm ID) packed with alumina. The column was eluted with acetonitrile (150 ml) saturated with hexane. The water sample (500 ml) was extracted with hexane (3 × 50 ml) and the extracts were analysed with a gas chromatograph (Shimadzu GC-7A, electron-capture detector); a glass column (2 m × 3 mm ID) packed with 3% OV-1 on 80/100 mesh Chromosorb WHP was employed. Operating temperatures were, for both injection port and detector, 270°C and for the column, 195°C. Nitrogen served as a carrier gas. Recovery of herbicide was determined by adding herbicide (4·5 μg) individually to fish samples, mixing with distilled water (500 ml) and then analysing as above. The average recoveries from fish samples were 90, 89 and 92% and from water samples were 92, 91 and 94% for butachlor, thiobencarb and chlomethoxyfen, respectively.

2.4 Determination of rate constants

The uptake and depuration in fish of a chemical from water was described by Branson *et al.*,[12] as following a reversible reaction with a one-compartment model (model 1):

$$C_1 \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} C_f$$

k_{12}: uptake rate constant (ml g^{-1} h^{-1})
k_{21}: depuration rate constant (h^{-1})
C_1: chemical concentration in water
C_f: chemical concentration in fish

during accumulation

$$C_f = (k_{12}/k_{21})C_1(1 - e^{-k_{21}t}) \quad (1)$$

and during elimination

$$C_f = A\, e^{-k_{21}t} \quad (2)$$

A: final chemical concentration in fish after dosing

The bioconcentration factor (BCF) under steady-state conditions was

$$BCF = C_f/C_1 = k_{12}/k_{21}$$

Könemann and Leeuwen[13] developed a two-compartment model (model 2) to describe the kinetics of chemicals, taking into consideration metabolism and excretion as follows:

$$C_1 \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} q_1 \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} q_2$$

$$\downarrow$$

$$= C_f \times \text{(fish mass)}$$

k_{12}: uptake rate constant
k_{21}: depuration rate constant
k_{23}, k_{32}: transfer rate constant
q_1, q_2: amounts of chemicals of compartments 1 and 2, respectively, in fish

during accumulation:

$$C_f = A_1(1 - e^{-a_1t}) + A_2(1 - e^{-a_2t}) \quad (3)$$

Whereas during elimination[14]

$$C_f = A'_1\, e^{-a_1t} + A'_2\, e^{-a_2t} \quad (4)$$

3 RESULTS AND DISCUSSION

The concentrations selected as nominal and the actual concentrations of the three herbicides in water at high and low levels are specified in Table 1. No difference was found between the nominal and measured concentration in the low-exposure experiment, but at high-concentration exposure the actual concentrations are lower than nominal concentrations. LC_{50} values of herbicides to black silver carp were measured in our previous work.[15] As chlomethoxyfen is only slightly soluble in water, its LC_{50} value was not determined, but at a concentration of 1·78 mg litre^{-1} obtained by using a small amount of emulsifier, the chemical showed no biological activity against black silver carp.[15]

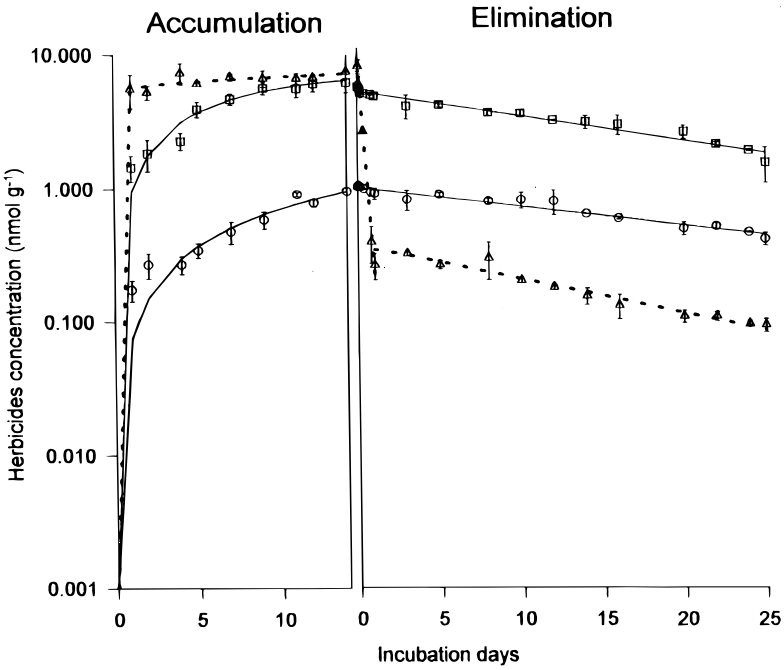

Fig. 1. Accumulation and elimination of (△) chlomethoxyfen, (○) butachlor and (□) thiobencarb in black silver carp during exposure to low concentration for 14 days and removal to clean water for clearance. (——) Simulation with model 1, (- - -) Simulation with model 2.

The accumulation of herbicides in black silver carp during exposure for 14 days and elimination after transfer to clean water are shown in Fig. 1 (low concentration) and Fig. 2 (high concentration).

In model 1 (one-compartment), k_{12} and k_{21} were determined by fitting experimental data for herbicide concentration in fish (C_f) (C_1 is constant) into eqns (1) and (2). In the steady state, BCF is equal to C_f/C_1 or k_{12}/k_{21}. The rate constants k_{12} and k_{21} and the BCF from simulation and measurement are shown in Table 2.

Mackay and Hughes[16] reported that non-polar chemicals of low water solubility and high hydrophobicity are readily absorbed by fish. Among the three herbicides, butachlor gave the lowest BCF in fish at 14 days although its water solubility is higher than that of

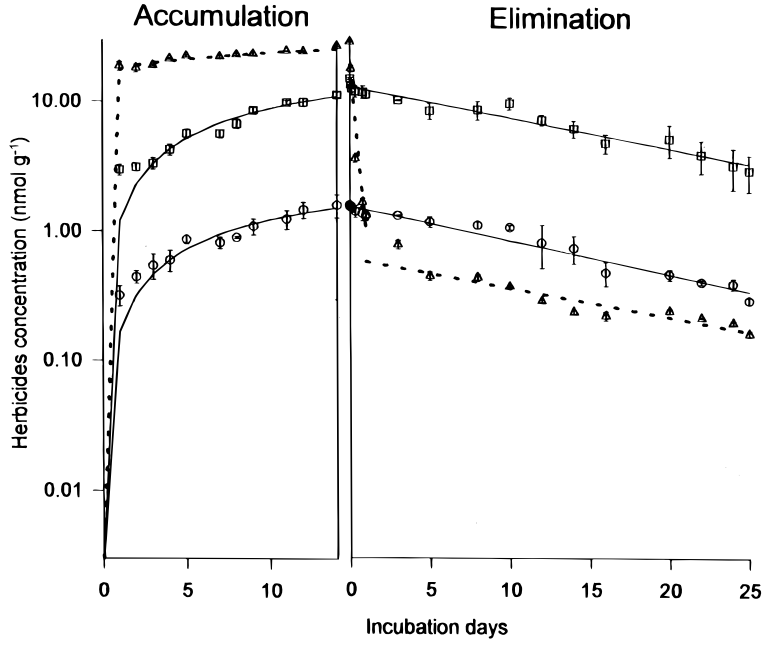

Fig. 2. Accumulation and elimination of (△) chlomethoxyfen, (○) butachlor and (□) thiobencarb in black silver carp during exposure to high concentration for 14 days and removal to clean water for clearance. (——) Simulation with model 1, (- - -) Simulation with model 2.

TABLE 2
Rate Coefficients for Uptake (k_{12}) and Elimination (k_{21}) and BCF Calculated from Exposure for 14 Days with Model 1

			BCF		
			At day 14		*In the steady state*
Herbicides	k_{12}	k_{21}	*Measured* (C_f/C_1)	*Stimulated from eqn (1)*	*Predicted* (k_{12}/k_{21})
Low concentration					
Butachlor	38·6 (±5·4)	0·017 (±0·006)	480·5	480·8	2721 (±1278)
Thiobencarb	257·7 (±14·2)	0·13 (±0·014)	1772·1	1656·6	2017 (±327)
Chlomethoxyfen	1475 (±178·8)	1·52 (±0·19)	1077·2	968·7	1001 (±242)
High concentration					
Butachlor	18·7 (±2·3)	0·07 (±0·018)	178·9	169·9	247 (±59)
Thiobencarb	76·9 (±7·2)	0·06 (±0·015)	714	695	1399 (±469)
Chlomethoxyfen	476·3 (±86·3)	1·21 (±0·23)	468·0	390·3	422 (±141)

TABLE 3
Simulated and Measured Concentrations of Herbicides in Fish after Exposure to Herbicide for 34 Days

	C_f (*μmol kg*$^{-1}$ *of fish*)		
	Simulation		
Herbicides	*Model 1*	*Model 2*	*Measured*
Low concentration			
Butachlor	1·99	—	1·52 (±0·03)
Thiobencarb	7·81	—	8·11 (±0·11)
Chlomethoxyfen	—	8·25	8·02 (±0·16)
High concentration			
Butachlor	2·19	—	2·55 (±0·18)
Thiobencarb	16·32	—	18·15 (±0·52)
Chlomethoxyfen	—	30·92	32·12 (±0·20)

chlomethoxyfen; this is attributed to its lower rate-constant for uptake (k_{12}). The steady state was attained after exposure in chlomethoxyfen for 14 days, but for butachlor and thiobencarb it was difficult to attain the steady state, especially for the former (Table 2). By longer exposure, BCF increased significantly for butachlor at low concentration; due to the lower k_{21}. Butachlor is thus accumulated in black silver carp and difficult to eliminate. Chlomethoxyfen showed a greater uptake and depuration rate coefficient than butachlor and thiobencarb (Figs 1 and 2). A steep slope for chlomethoxyfen ($a_1 = 2{\cdot}96$; $a_2 = 0{\cdot}05$) much exceeded those for butachlor ($a_1 = 0{\cdot}06$) and thiobencarb ($a_1 = 0{\cdot}05$) at a high concentration of herbicides. At a low concentration, chlomethoxyfen showed $a_1 = 3{\cdot}70$ and $a_2 = 0{\cdot}06$, butachlor showed $a_1 = 0.03$, and thiobencarb showed $a_1 = 0{\cdot}04$. No significant depuration rate (a_2) of a

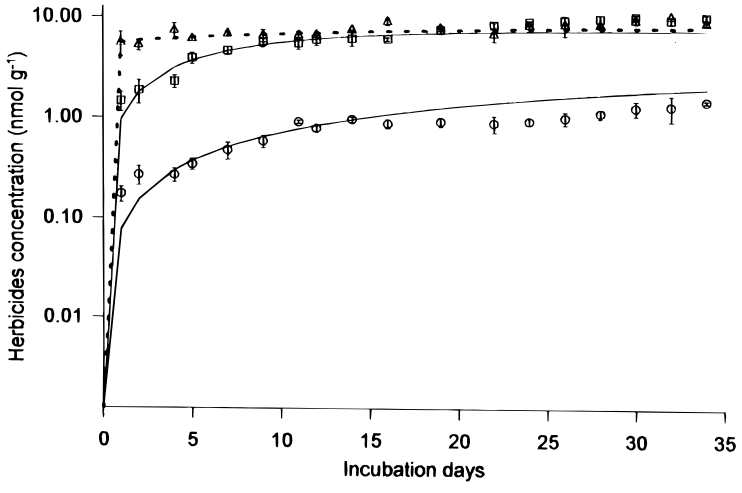

Fig. 3. Measured and predicted accumulation of (△) chlomethoxyfen, (○) butachlor and (□) thiobencarb in black silver carp during exposure to low concentration for 34 days. (——) Simulation with model 1, (- - -) Simulation with model 2.

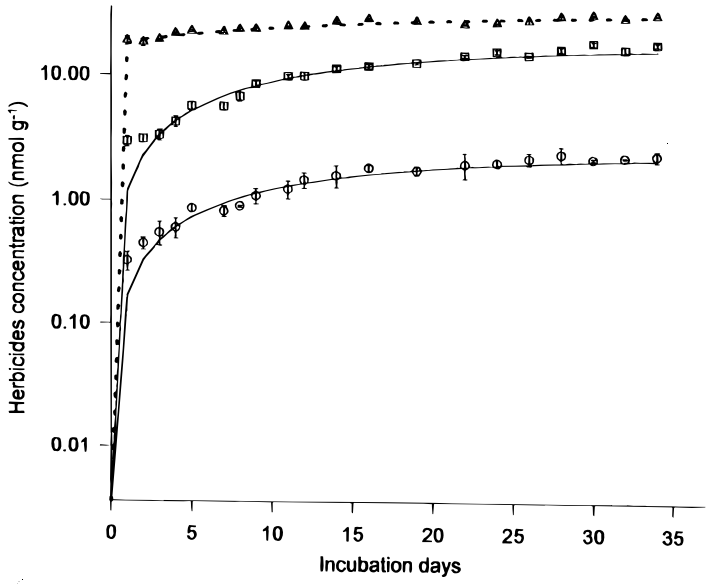

Fig. 4. Measured and predicted accumulation of (△) chlomethoxyfen, (○) butachlor and (□) thiobencarb in black silver carp during exposure to high concentration for 34 days. (——) Simulation with model 1, (- - -) Simulation with model 2.

TABLE 4
Uptake (k_{12}), Elimination (k_{21}) and Transfer (k_{23} and k_{32}) Rate Coefficients for Chlomethoxyfen with Model 2

	k_{12}	k_{21}	k_{23}	k_{32}	BCF $[(A_1 + A_2)/C_1]$
Low conc.	1122 (±235)	3·52 (±0·73)	0·17 (±0·034)	0·06 (±0·014)	1339 (±281)
High conc.	363·2 (±55·3)	2·76 (±0·56)	0·22 (±0·038)	0·06 (±0·02)	575 (±107)

second compartment was shown for thiobencarb and butachlor in black silver carp. Thus, the accumulation and elimination kinetics of butachlor and thiobencarb could be simulated with model 1 (one-compartment) and of chlomethoxyfen with model 2 (two-compartment) as shown in Figs 1 and 2. The biotic half-lives of butachlor, thiobencarb and chlomethoxyfen in black silver carp, calculated from (ln 2)/a_1, were 11·6 and 23·1 days, 13·9 and 17·3 days and 5·6 and 4·5 h for high and low concentrations, respectively. The parameters on the basis of the accelerated test (exposure for 14 days) were used to predict the 34-day exposure, shown in Table 3. The predictions with model 1 based on the accelerated test fitted well with the observed data for butachlor and thiobencarb during the test for 34 days, but not for chlomethoxyfen. However, using model 2, the concentration of chlomethoxyfen in fish fitted well with the measured value (Table 3).

Residues of the three herbicides in black silver carp during exposure for 34 days, measured from the experimental results and simulated with the above parameters, are shown in Figs 3 and 4. During exposure at both low and high concentrations, the transfer rate coefficient k_{23} was apparently larger than k_{32} for chlomethoxyfen (Table 4); k_{23} and k_{32} were not calculated for exposure to butachlor and thiobencarb, as a single-compartment model was adequate. The value of k_{21} for butachlor and thiobencarb was small (Table 2), but the decrease of q_1 was similarly slow (so there was no movement to q_2), and hence the one-compartment model is adequate to describe their accumulation and elimination kinetics. However, in the case of chlomethoxyfen, because $k_{21} \gg k_{23}$ or k_{32}, q_1 decreased quickly, and q_2 was obviously distinguished by large k_{23} and small k_{32}, so the two-compartment model is necessary to describe the kinetics.

4 CONCLUSIONS

In relation to physiology, the two-compartment model serves to distinguish another compartment (the second compartment) from the active compartment (the first compartment). The active compartment is used for metabolism and elimination. In the elimination experiment, chlomethoxyfen concentration in fish decreased steeply during the early period and then slowly in the later period, i.e. the herbicide was cleared rapidly from the first compartment (the active compartment) and relatively slowly from the second compartment, but for butachlor and thiobencarb clearance from the first compartment was slow, and hence the effect of a second compartment was not detected.

The two-compartment model ought to be better than the one-compartment model to describe and to predict the accumulation and elimination kinetics of chemicals in fish, but was suitable only for chlomethoxyfen in this work.

ACKNOWLEDGEMENT

We thank the Council of Agriculture of Taiwan, 79-Agr-7.1-Fish-13(3) for support.

REFERENCES

1. Beestman, G. B. & Deming, J. M., Dissipation of acetanilide herbicides from soil. *Agro. J*., **66** (1974) 308–11.
2. Chen, Y. L. & Chen, J. S., Degradation and dissipation of herbicide butachlor in paddy field. *Nihon Noyaku Gakkaishi* (*J. Pestic. Sci.*), **4** (1979) 431–8.
3. Ishikawa, K., Nakamura, Y. & Kuwatsuka, S., Degradation of benthiocarb herbicide in soil. *Nihon Noyaku Gakkaishi* (*J. Pestic. Sci.*), **1** (1976) 49–57.
4. Yusa, Y. & Ishikawa, K., Disappearance of benthiocarb herbicide in irrigation water. *Proc. 6th Asian-Pacific Weed Sci. Soc. Conf*., (1977) 596–602.
5. Chiang, H. C., Wang, Y. S. & Chen, Y. L., Residues and dissipation of three major herbicides, butachlor, chlomethoxynil and benthiocarb in paddy fields in Taiwan. *Proc. 11th Asian-Pacific Weed. Sci. Soc. Conf*., (1987) 163–71.
6. Yamagishi, T. & Akiyama, K., 1,3,5-Trichloro-2-(4-nitrophenoxy) benzene in fish, shellfish and sea water in Tokyo Bay, 1977–1979. *Arch. Environ. Contam. Toxicol*., **10** (1981) 627–35.
7. Ohyama, T., Jin, K., Katoh, Y., Chiba, Y. & Inoue, K., 1,3,5-Trichloro-2-(4-nitrophenoxy) benzene (CNP) in water, sediments, and shellfish of the Ishikari River. *Bull. Environ. Contam. Toxicol*., **37** (1986) 344–7.
8. Ohyama, T., Jin, K., Katoh, Y., Clkliba, Y. & Inoue, K., Fate and behavior of herbicides, butachlor, CNP, chlomethoxynil, and sediments of the Ishikari River. *Bull. Environ. Contam. Toxicol*., **39** (1987) 555–62.
9. Neely, W. B., Estimating rate constants for the uptake and clearance of chemicals by fish. *Environ. Sci. Technnol*., **13** (1979) 1506–10.
10. Connolly, J. P., Application of a food chain model to polychlorinated biphenyl contamination of the lobster and winter flounder food chains in New Bedford harbor. *Environ. Sci. Technol*., **25** (1991) 760–70.
11. Bruggeman, W. A., Martron, L. B. J. M., Kooiman, D. & Hutzinger, O., Accumulation and elimination kinetics of di-, tri- and tetrachlorobiphenyls by goldfish after dietary and aqueous exposure. *Chemosphere*., **10** (1981) 811–32.
12. Branson, D. R., Blau, G. E., Alexander, H. C. & Neely, W. B., Bioconcentration of 2,2′4,4′-tetrachlorobiphenyl in rainbow trout as measured by an accelerated test. *Trans. Am. Fish. Soc*., **104** (1975) 785–91.
13. Könemann, H. & Leeuwen, K. V., Toxicokinetics in fish: accumulation and elimination of six chlorobenzenes by guppies. *Chemosphere*, **9** (1980) 3–19.
14. Spacie, A. & Hamelink, J. L., Alternative models for describing the bioconcentration of organics in fish. *Environ. Toxicol. and Chem*., **1** (1982) 309–20.
15. Wang, Y. S., Jaw, C. G., Tang, H. C., Lin, T. S. & Chen, Y. L., Accumulation and release of herbicides butachlor, thiobencarb and chlomethoxyfen by fish, clam and shrimp. *Bull. Environ. Contam. Toxicol*., **48** (1992) 474–80.
16. Mackay, D. & Hughes, A. I., Three-parameter equation describing the uptake of organic compounds by fish. *Environ. Sci. Technol*., **18** (1984) 439–44.
17. Hisada, T. & Imamura, S. (eds), *Short Review of Herbicides*. Hodogaya Chemical Co. Ltd, Tokyo, Japan, 1982, pp. 34–5.
18. Tomlin, C. (ed.), *The Pesticide Manual, 10th* edn. The British Crop Protection Council, Farnham, Surrey, UK. 1994, p. 979–80.
19. Meister, R. T. (ed.), *Farm Chemicals Handbook*. Meister Publishing Company, Ohio, USA, 1993, pp. c73–4.